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Bioefficacy of a mixed biocide BtA against *Helicoverpa armigera* (Lepidoptera: Noctuidae) and its contact toxicity to pupae and adults of parasitoid *Microplitis mediator* (Hymenoptera: Braconidae)

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Abstract: To scientifically utilize biocides and conserve natural enemies for integrated control of *Helicoverpa armigera*, the bioefficacy of a mixed biocide BtA in comparison with seven commercial insecticides (abamectin, Bt toxin, beta-cypermethrin, phoxim, chlorfluazuron, fenvalerate and carbosulfan) against *H. armigera* and its contact toxicity to pupae and female adults of parasitoid *Microplitis mediator* were bioassayed in the laboratory and compared. Toxicity of these compounds on the 3rd instar larvae of *H. armigera* by leaf dip bioassays within 72 h in the laboratory showed that BtA had high toxicity (LC_{50} = 0.7364 mg/mL) in comparison with the other commercial insecticides. Mortalities of the 3rd instar larvae of *H. armigera* exposed to *Brassica rapa* var. *pekinensis* treated with 4 mg/mL of these compounds were significantly different at 24, 48, and 72 h in pot trials ($P \leqslant 0.05$). BtA had no significantly different bioefficacy ($P > 0.05$) in the experimental pots from the other commercial insecticides against *H. armigera* larvae at 72 h after treatment. After application of BtA, larval mortality of *H. armigera* increased as time extended. Bioassay of the contact toxicity of these compounds to pupae and female adults of parasitoid *M. mediator* showed that BtA has low toxicity to pupae and female adults of *M. mediator*, with the mortality values of 13.82% and 7.33%, respectively, in comparison with fenvalerate and carbosulfan. We so concluded that BtA has a moderate toxicity to the lepidopterous pest, *H. armigera*, and a relatively low toxicity to its parasitoid, *M. mediator*.

Key words: *Helicoverpa armigera*; *Microplitis mediator*; insecticides; biocide; BtA; toxicity; contact toxicity

1 INTRODUCTION

The order Lepidoptera and more specifically the family Noctuidae comprise a large number of destructive crop pests including the bollworm, *Helicoverpa armigera* (Hübner). *H. armigera* is a serious pest of cotton, maize, and many other crops in China (Guo, 1997). A broad complex of natural enemies acts against *H. armigera* in various agro-ecosystems worldwide (Stam and Elmosa, 1990; Kuklinski and Borgemeister, 2002). Although conventional synthetic pesticides have been widely applied to control *H. armigera*, integrated pest management (IPM) strategies including the use of natural enemies are needed.

A beneficial braconid wasp has been shown to be an efficient biological control agent for *H. armigera*. The parasitoid seems to be especially efficient natural enemy, and has been reported to cause mortality levels up to 50% of *Heliothis* spp. in cotton (King *et al.*, 1985). In China, several parasitoid species attack *H. armigera* in the field, one of which is the braconid wasp *Microplitis mediator* (Haliday). This parasitoid is a solitary larval endoparasitoid, widely distributed in the Palaearctic region (Slovak, 1985; Arthur and Mason, 1986; Mason *et al.*, 2001). It is polyphagous, using ~40 different pest lepidopterous larvae including some noctuids and geometrids as hosts (Arthur and Mason, 1986; Wang and Hun, 1992). It is also an important natural enemy of *H. armigera* in northern China (Liu *et al.*, 2005; Wu and Guo, 2005; Li *et al.*, 2006a). This parasitoid

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prefers the 1st and 2nd instar larvae of *H. armigera*. It achieved the parasitism rate as high as 22.6% on *H. armigera* in the cotton fields in Hebei Province in 1982 (Wang *et al.*, 1984). The technology necessary for the mass propagation of the parasitoid has been developed since 2002 in China, and releasing of wasps of this parasitoid to control *H. armigera* in cotton fields in Xinjiang had shown promising results (Li *et al.*, 2006b).

A new multiple-toxin, mixed biocide BtA, *Bacillus thuringiensis* var. *kurstaki* mixed with abamectin produced by fermentation of *Streptomyces avermitilis*, was formulated, in order to expand microbial toxicity to more target pests (Liu and Sengonca, 2000; Sengonca and Liu, 2001b). Efficacy of BtA in controlling vegetable pests in different insect orders has been demonstrated (Sengonca *et al.*, 2001) and compared with the other commercial insecticides, BtA was relatively harmless against parasitoids, such as *Apantales plutellae* Kurdj. (Hymenotpera: Braconidae) (Sengonca and Liu, 2001a) and *Microplitis mediator* (Haliday) (Hymenotpera: Braconidae) (Wanna *et al.*, 2010), and predators, such as *Amblyseius longispinosus* (Evans) (Acari: Phytoseiidae), *Erigonidium graminicola* (Sundv.) (Araneida: Linyphiidae), *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) (Liu and Sengonca, 2002; Sengonca and Liu, 2003), *Orius strigicollis* Poppius (Heteroptera: Anthocoridae) and *Stethorus cantonensis* Pang (Coleoptera: Coccinellidae) (Zhu *et al.*, 2006). BtA has been successfully used in an integrated cabbage pest management program in Fuzhou, China (Sengonca and Liu, 2002, 2003), where it showed high efficacy in reducing pest abundance with little or no harm to their natural enemies (Sengonca and Liu, 2003). BtA has overcome the disadvantages of *B. thuringiensis* by possessing toxicity against a broader range of pests and a relatively rapid killing speed better than *B. thuringiensis*. The objective of this study was therefore to evaluate the bioefficacy of the mixed biocide BtA against *H. armigera* and its toxicity to pupae and adults of *M. mediator*.

2 MATERIALS AND METHODS

2.1 Test insects

Pupae of *H. armigera* were obtained from Zhejiang Agriculture and Forestry University (ZAFU), China. The mass rearing of *H. armigera* larvae was conducted using the artificial diet of Shen and Wu (1995) in the laboratory at ZAFU. They were reared under ambient laboratory conditions at 25 ± 1℃, 75% ± 10% RH, and a photoperiod of 16L: 8D. Variable numbers of 1st and 2nd instar larvae were reared together in 570 mL clear plastic containers on artificial diet for about 1 week to allow the larvae to reach the 3rd instar. They were then transferred singly into 28 mL clear plastic containers (4 cm in height and 7 cm in diameter) containing ~14 mL of diet, in which they were held until pupation. Pupae were removed from the cups, sterilized in 0.25% sodium hypochlorite solution, rinsed with distilled water, sexed and transferred into a clear container lined with paper. Three to five pairs of moths were placed in each clear container. Adult moths emerged directly into a mating cage (40 cm × 25 cm × 25 cm) and were supplied with 10% honey solution as food. Upon emergence, the moths were allowed 3 – 4 d for mating and egg maturation. Eggs laid on the paper tubes were removed by cutting the paper into small pieces, and placed in 570 mL clear plastic containers. The eggs were collected daily by changing the paper and kept in a container till they hatched. After hatching, larvae were fed on artificial diet and mass reared under the standard rearing conditions described above. Insecticide bioassays were performed on the 3rd instar larvae.

The parasitoid *M. mediator* was obtained from the Institute of Plant Protection, Hebei Academy of Agriculture and Forestry, China. Parasitoids were reared at 26 ± 1℃ with a photoperiod of 14L: 10D in the laboratory. The adults were paired, and each pair was put in a glass vial sealed with a cotton plug. A cotton wool pellet wet with a small drop of 10% honey solution was put into the vial to serve as food. The mated female wasps were transferred to parasitize *H. armigera* larvae and the parasitized larvae of *H. armigera* were reared on artificial diet. Toxicity bioassays were conducted on pupae and female adults.

2.2 Mixed biocide of BtA

In all the bioassay procedures, the self-prepared biocide BtA was tested, in which *Bacillus thuringiensis* var. *kurstaki* 16 000 IU/mg (Fujiang Pucheng Green Shell Biological Technology Co., Ltd.), and abamectin 0.18% EC (Zhejiang Shenghua Biok Biological Co., Ltd.) were manufactured by Sendebao Bioproducts Co., Ltd.

2.3 Commercial insecticides

Seven commercial insecticides used in these tests were trade grade insecticides and formulations: *Bacillus thuringiensis* var. *kurstaki* (Bt toxin) 16 000 IU/mg (Lesi Chemical Factory), beta-cypermethrin 45% ME (Shenzhen Ruide-Feng

Pesticide Factory), phoxim 40% EC (Lianyungang Liben Pesticide Factory), chlorfluazuron 50 g a. i./L (Zhejiang Jinniu Pesticide Factory), fenvalerate 20% EC (Hangzhou Qingfeng Agricultural Chemistry Factory), and carbosulfan 200 g a. i./L (Suzhou Fumeishi Pesticide Factory).

2.4 Leaf disc bioassay of toxicity against *H. armigera*

The concentrations of the mixed biocide BtA and seven commercial insecticide formulations were diluted in distilled water to achieve the desired concentrations; 5 concentrations with 3 replications for pre-test, and 6 concentrations with 5 replications for final-test. Toxicity bioassays were conducted at 25 ± 1℃, 75% ± 10% RH, and a photoperiod of 16L: 8D, at Institute of Insect Science, Zhejiang University (IISZJU), Hangzhou, China, by leaf residue bioassay. Leaf discs (2.5 cm in diameter) cut from Chinese cabbage leaves with a cork borer, were dipped into the treatment solutions for 10 s with gentle agitation and dried in the air for 1 h at room temperature. Each concentration consisted of twenty-five Petri dishes. Four leaf discs were transferred into each Petri dish (2 cm in height and 9 cm in diameter). Five the 3rd instar larvae of *H. armigera* were released alongside the leaf discs in each Petri dish. The same number of leaf discs per treatment was dipped into distilled water as the control. Moistened filter papers were placed beneath the leaf discs to avoid desiccation. After releasing the larvae, test containers were covered with a piece of black cloth to minimize cannibalism. The whole experiment was repeated twice. Larval mortality was recorded at 72 h after treatment. Treatments where mortality in the control exceeded 20% were discarded and repeated.

2.5 Pot trial bioassay of toxicity against *H. armigera*

Pot trials were conducted at IISZJU in June 2009. Chinese cabbages, *Brassica rapa* var. *pekinensis*, were grown under local management practices from seeds in seedling trays. After 2 weeks, seedlings were transferred into 2 L plastic pots (20 cm in height and 23 cm in diameter) and grown until 6 week-old for use. A Chinese cabbage field was divided into 4 experimental pots with 2 pots each along the vertical and horizontal axes. A randomized complete block design was used in 4 replications. BtA and seven commercial insecticide formulations were tested with a recommended field rate for against *H. armigera* at the concentration of 4 mg/mL. The various treatments were applied using spray equipment and misting the foliage to run-off. Tap water was used as the control. The spray equipment was drained and triple rinsed after each treatment to avoid cross contamination. Five the 3rd instar larvae of *H. armigera* were placed on each plant. Pots were covered by net cages size 16 cm × 16 cm × 30 cm. Larval mortalities were measured at 24, 48, and 72 h after treatment.

2.6 Bioassay of toxicity to parasitoid *M. mediator*

Contact toxicity of *M. mediator* at the pupal stage was evaluated by direct-dip bioassay, a modified simulated method after Idris and Grafius (1993). BtA and seven commercial insecticide formulations were tested for bioassay at a concentration of 4 mg/mL. Bioassays were conducted at 25 ± 1℃, 75% ± 10% RH, and a photoperiod of 12L: 12D. Fifty pupae of *M. mediator* (2 day-old) were placed in a tea filter (4 cm × 4 cm) and dipped in the various treatments for 4 s. Pupae were dipped in distilled water as the control. Pupae were removed from a tea filter, blotted dry on filter paper towels, and transferred into a petri dish (15 mm in height and 7 cm in diameter). Pupal mortalities were recorded every 24 h until all the individuals were either emerged as adults or died. A completely randomized design was used in 3 replications.

Toxicity bioassay, modified from the methods of Idris and Grafius (1993), was used for female adult stage of *M. mediator*. BtA and seven commercial insecticide formulations were tested for bioassay at a concentration of 4 mg/mL. The various prepared treatments were poured into test tubes (18 cm in height and 15 mm in diameter) and swirled for 1 min. The excess quantity was poured off and the residue was air-dried for 2 h. Distilled water was used as the control. Ten newly emerged female adults of *M. mediator* were released in each treated tube. The test tube was plugged with a cotton plug. Diluted honey solution was supplied as food through the cotton plug. Experiments were replicated many times until the total number counted was 150 individuals of *M. mediator*. A completely randomized design was used. Mortalities of female adults of *M. mediator* were recorded at 24 h after treatment.

2.7 Data analysis

Toxicity bioassay, linear regression analysis was performed from the data obtained to estimate larval mortality for each concentration of BtA, seven commercial insecticides and the control (distilled water). The resulting concentration-larval mortality data was subjected to probit analysis (Finney, 1971). Pot trials, data collected from sampling in

the pot was analyzed to determine average number per 16 experimental pots in all 4 replications for each treatment. Larval mortality of the 3rd instar of *H. armigera* in control pots (tap water) were used to correct the larval mortality obtained with 4 mg/mL of treatments pots according to Abbott (1925). Mortalities of pupae and female adults of *M. mediator* in the controls were used to correct the pupal and female adult mortalities obtained with 4 mg/mL of treatments according to Abbott (1925). Data were analyzed by one-way analysis of variance (ANOVA) following by the least significant differences (LSD) for mean separation using SPSS (SPSS, 1998). Percentage data were transformed using the arcsine square root transformation and means presented were transformed from proportions to percentages.

3 RESULTS

3.1 Toxicity of BtA and seven commercial insecticides against *H. armigera* assayed with leaf discs

Toxicities (LC_{50}) of BtA, abamectin, Bt toxin, phoxim, chlorfluazuron, fenvalerate, and carbosulfan in comparison to distilled water and beta-cypermethrin to the 3rd instar larvae of *H. armigera* by leaf-dip bioassays in the laboratory were determined (Table 1). Abamectin was the most toxic commercial insecticide to *H. armigera* larvae at 72 h after treatment with the LC_{50} value of 0.0019 mg/mL compared with 0.1071 mg/mL of phoxim, 0.2218 mg/mL of BtA, 0.8849 mg/mL of Bt toxin, 1.0752 mg/mL of fenvalerate, 1.7167 mg/mL of carbosulfan, and 3.1351 mg/mL of beta-cypermethrin, respectively. The least effective compound on *H. armigera* larvae was 14.6249 mg/mL of chlorfluazuron.

3.2 Toxicity of BtA and seven commercial insecticides against *H. armigera* assayed with pot trials

Mortality values of the 3rd instar larvae of *H. armigera* exposed to *B. rapa* var. *pekinensis* treated with various treatments were significantly different at 24, 48, and 72 h ($F = 7.45$, $df = 8$, $P < 0.001$; $F = 22.35$, $df = 8$, $P < 0.001$; $F = 11.49$, $df = 8$, $P < 0.001$, respectively) (Table 2). Abamectin and phoxim residual efficacy against *H. armigera* larvae at 24 to 72 h after treatment resulted in larval mortality values between 40% and 70%. Larval mortality of *H. armigera* after application of BtA, fenvalerate, carbosulfan, and beta-cypermethrin increased as time extended. *H. armigera* larvae were primarily affected by chlorfluazuron and Bt toxin at 48 and 72 h after treatment, respectively. BtA at the concentration of 4 mg/mL significantly decreased the feeding activity of *H. armigera* larvae within 72 h. In addition, there was no significant difference in efficacy in the field between BtA and the other commercial insecticides against *H. armigera* larvae at 72 h after treatment. Therefore, properly timed treatments of BtA may be beneficial to prevent the damage from *H. armigera*.

3.3 Toxicity of BtA and seven commercial insecticides to *M. mediator*

Mortality values of *M. mediator* pupae at the time till pupae emerged within 24 h by direct-dip bioassay at 4 mg/mL of BtA and seven commercial insecticides are presented in Table 3. Significant difference was found among the treatments ($F = 79.47$, $df = 8$, $P < 0.001$). Fenvalerate had the highest contact toxicity to *M. mediator* pupae and it caused significantly higher mortality than the other commercial insecticides with the pupal mortality of 73.17%. BtA had significantly lower contact toxicity to *M. mediator* pupae with the mortality of 13.82% at the time till pupae emerged than beta-cypermethrin, fenvalerate, and carbosulfan. Bt toxin and chlorfluazuron had toxicity to *M. mediator* pupae, with mortality values not significantly different from the control.

Mortality values of female adults of *M. mediator* treated with BtA and seven commercial insecticides after 24 h treatment by contact toxicity bioassay are shown in Table 3. Mortalities of female adults were significantly different ($F = 3587.69$, $df = 8$, $P < 0.001$). Phoxim and carbosulfan had the highest contact toxicity to female adults of *M. mediator* with mortality values of 100% and caused significantly higher mortalities to female adults in comparison with the other commercial insecticides. BtA showed the lowest contact toxicity to female adults of *M. mediator* with mortality values less than 10% and significantly lower mortality to female adults compared with abamectin, beta-cypermethrin, phoxim, fenvalerate and carbosulfan. Bt toxin and chlorfluazuron did not show contact toxicity to female adults of *M. mediator* within 24 h. Results indicated that BtA had low toxicity to pupae and female adults of *M. mediator* in comparison with beta-cypermethrin, fenvalerate, and carbosulfan in the laboratory test.

Table 1 Toxicities of BtA and seven commercial insecticides to the 3rd instar larvae of *Helicoverpa armigera* at 72 h after treatment assayed by using leaf disc method in the laboratory

Treatments	Slope (± *SE*)	LC_{50} (mg/mL)	95% CL
BtA	2.15 ± 0.80	0.2218	0.0660 – 0.3775
Abamectin	91.51 ± 1.03	0.0019	0.1940 – 0.1977
Bt toxin	2.08 ± 0.76	0.8849	0.7524 – 1.0173
Phoxim	25.20 ± 1.63	0.1071	0.1335 – 0.3477
Chlorfluazuron	0.10 ± 0.94	14.6249	14.4508 – 14.7989
Fenvalerate	0.25 ± 1.56	1.0752	0.8473 – 1.3031
Carbosulfan	1.20 ± 1.00	1.7167	0.5522 – 1.8812
Beta-cypermethrin	0.24 ± 1.52	3.1351	2.9064 – 3.3637

Number of *H. armigera* larvae in the probit analysis is 150. For the preceding LC estimate, 95% confidence limits were based on probit analysis.

Table 2 Toxicities of BtA and seven commercial insecticides to the 3rd instar larvae of *Helicoverpa armigera* assayed by using pot trials

Treatments	Mortalities (%) at different hours after treatment		
	24 h	48 h	72 h
BtA	8.02 ± 9.84 ab	34.66 ± 3.17 b	55.52 ± 5.77 bc
Abamectin	50.00 ± 3.33 d	70.50 ± 3.66 d	70.50 ± 3.66 c
Bt toxin	0.00 ± 0.00 a	0.00 ± 0.00 a	34.66 ± 3.17 b
Phoxim	39.52 ± 4.94 cd	60.48 ± 4.94 cd	70.50 ± 3.66 c
Chlorfluazuron	0.00 ± 0.00 a	5.28 ± 7.67 a	55.03 ± 2.88 bc
Fenvalerate	15.38 ± 8.26 bc	5.03 ± 2.88 bcd	71.43 ± 11.11 c
Carbosulfan	15.38 ± 8.26 bc	39.52 ± 4.94 bc	55.52 ± 5.77 bc
Beta-cypermethrin	15.38 ± 8.26 bc	34.19 ± 5.82 b	55.03 ± 2.88 bc
Tap water	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a

Data (mean ± *SE*) within a column followed by different letters are significantly different ($P < 0.05$; LSD test). Data are adjusted to Abbott's formula and transformed by arcsine for proportions. The same for Table 3.

Table 3 Toxicities of BtA and seven commercial insecticides to pupae at the time till pupae emerged within 24 h and female adults at 24 h after treatment of *Microplitis mediator* assayed by using direct-dip bioassay in the laboratory

Treatments	Pupal mortalities (%)	Mortalities of female adults (%)
BtA	13.82 ± 6.14 b	7.33 ± 1.15 b
Abamectin	13.01 ± 9.86 b	44.00 ± 2.00 c
Bt toxin	0.00 ± 0.00 a	0.00 ± 0.00 a
Phoxim	17.07 ± 6.45 b	100.00 ± 0.00 f
Chlorfluazuron	0.00 ± 0.00 a	0.00 ± 0.00 a
Fenvalerate	73.17 ± 6.45 e	80.67 ± 1.15 e
Carbosulfan	41.46 ± 0.00 d	100.00 ± 0.00 f
Beta-cypermethrin	27.64 ± 1.41 c	71.33 ± 4.62 d
Distilled water	0.00 ± 0.00 a	0.00 ± 0.00 a

4 DISCUSSIONS AND CONCLUSIONS

4.1 Comparison of toxicity of various insecticides to *H. armigera*

Higher slope in concentration-mortality regression lines indicated a smaller variation in the response of individuals within a population to a certain compound (Zanuncio *et al.*, 1998). The slope of abamectin curve for *H. armigera* was the highest, indicating a homogeneous response of this pest to abamectin. In this case, a small variation in concentration led to a big variation in the pest mortality (Zanuncio *et al.*, 1998). In contrast, chlorfluazuron curve gave a small slope for *H. armigera*, indicating a low probability of toxicity of this insecticide against this species in that high insecticidal concentrations are applied in the field.

Slope of BtA curve for *H. armigera* tested was over 2 compared to that of other chemical insecticides below 1 (Table 1), showing that BtA was more toxic to this pest than Bt toxin, fenvalerate, carbosulfan, beta-cypermethrin, and chlorfluazuron.

Through cluster analysis, the toxicity levels were divided into 5 groups. The first group, only abamectin showed the highest toxicity level to *H. armigera*. The second group, BtA and phoxim showed high toxicity levels. BtA had been reported to be used effectively against the arthropod pests of Acari, Diptera, Homoptera, Coleoptera and Lepidoptera as well as to have comparatively less toxicity to natural enemies in comparison to chemical insecticides (Sengonca and Liu, 2001b, 2002, 2003; Sengonca *et al.*, 2001, 2006; Liu *et al.*, 2005). In this study, BtA also showed high toxicity to the lepidopterous pest, *P. xylostella*, and this is in accordance with the previous work of Liu and Sengonca (2002). The third group, beta-cypermethrin, carbosulfan, and fenvalerate showed moderate toxicity levels. The fourth group, chlorfluazuron showed low toxicity level. The fifth group, only Bt toxin showed the lowest toxicity to the pest.

4.2 Residual efficacy of various insecticides against *H. armigera*

Pot trials showed that abamectin was the most effective commercial insecticide to reduce damage caused by *H. armigera* by increasing larval mortality. A disadvantage of abamectin was reported by Pfeiffer (1985) that abamectin was an effective acaricide against the tetranychid pest, *Panonychus ulmi* (Koch), and had no significant effect on the predacious phytoseiid mite, *Panonychus ulmi* (Garman), although abamectin initially reduced predator densities when used in a full-season program. Banhawy and Bagoury (1985) reported that the activity of abamectin residues had lasted longer against the eriophyid pest than against the phytoseiid predator. Toxicity of abamectin was lower to the phytoseiid predator than to the tetranychids pest. However, survival and fecundity of the predator was reduced at the recommended field rates. Grafton *et al.* (1983) found that larvae of the predacious mite *Typhlodromus occidentalis* were more susceptible to abamectin than the adult females. Wu and Guo (2000) investigated that the field populations of the bollworm, *H. armigera*, had 50.34 – 90.93 times higher resistance to lamda-cyhalothrin in the samples collected from 16 locations in China from 1994 – 1997 as compared with the susceptible *H. armigera* population. They further reported their harm on the natural enemies too. The pests could quickly develop resistance to insecticides, while their natural enemies remained susceptible to them at the recommended doses applied in fields and gardens (Diraviam and Viraktamath, 1993; Forti and Loriatti, 1997). However, the application rate at which these insecticides have been applied should be reduced when they have been used in conjunction with biological control agents (Nakagawa, 1989; Cui and Xia, 1998).

Bt toxin showed obvious lethal effects on *H. armigera* within 72 h in this study. Also, Heimpel and Angus (1959) described *B. thuringiensis* as exhibiting variable modes of action, and thus categorized as host species, which included the majority of Lepidoptera, and were characterized by a cessation of feeding following by toxin ingestion, a paralysis restricted to the gut, and death within 2 to 4 days. Asano *et al.* (1976) reported that the application of Bt toxin preparations (Thuricide® HPSC and E-61®) highly suppressed the feeding of *Dendrolimus spectabilis*, even though no mortality occurred within 24 h of ingestion of treated foliage. The results obtained from the leaf disc bioassay indicated that beta-cypermethrin, phoxim, fenvalerate, and carbosulfan acted as contact poison since larvae did not feed on treated leaves. Chlorfluazuron has been used as an insect growth regulator inhibiting chitin synthesis, which is active against larval development in Lepidoptera. The results showed that during the affected stage of larval development in *H. armigera*, chlorfluazuron inhibited biosynthesis of chitin and resulted in abortive molting. It has a relatively long half-life in insect bodies with slow metabolism and elimination rate (Fahmy and Miyata, 1992; Sammour *et al.*, 2008). Mode of actions of Bt toxin and abamectin have previously been documented, *e. g.*, Bt toxin acted as a stomach poison (Chattopadhyay *et al.*, 2004), while abamectin acted as a stomach poison as well as a contact poison (Lasota and Dybas, 1990; Wehner *et al.*, 1993). Abro *et al.* (1988) stated that at 96 h, LC_{50} of abamectin against larvae of a laboratory strain of *P. xylostella* were 0.96 ng/larva and 0.041 μg/mL in topical application and residual ingestion bioassay. These are presumed to explain the mode of action of BtA since Bt toxin and abamectin are ingredients in BtA. Bt toxin poses few risks to the environment, however its toxicity is restricted primarily to lepidopterans that consume it, and most farmers prefer faster acting toxins that kill a wider range of pests (Aronson *et al.*, 1986;

Pietrantonio *et al.*, 1993). Two effective functions of Bt toxin as well as abamectin persisted, and the biocide demonstrated higher efficiency in controlling the insects than Bt toxin alone. The killing speed of BtA to the 3rd instar *H. armigera* was more rapid than Bt toxin alone and on the other hand, BtA killed target insects from Lepidoptera. The effectiveness of BtA has also been obtained by toxicity bioassay.

4.3 Toxicity of various insecticides to parasitoid *M. mediator*

In the present study of contact toxicity, treatment with BtA at the concentration of 4 mg/mL caused low contact toxicity both to *M. mediator* pupae at the time till pupae emerged and to female adults of *M. mediator* within 24 h after treatment. Female adults of *M. mediator* showed behavioral responses to insecticides by autotomizing their metathoraic legs at the trochanter-femur joint, after walking on surfaces coated with insecticide residues. BtA caused pupal and female adult mortalities less than 15% and 10%, respectively, while abamectin, phoxim, fenvalerate, carbosulfan, and beta-cypermethrin, resulted in pupal and female adult mortalities from 13% – 74% and 44% – 100%, respectively. Bt toxin and chlorfluazuron did not show obvious contact toxicity to pupae and female adults of *M. mediator*. Also, Talekar and Yang (1991) indicated that insect growth regulator (IGR) of teflubenzuron and Bt toxin were not toxic to adults of parasitoid *A. plutella*.

The results of this study indicated that synthetic chemical insecticides of pyrethroids (beta-cypermethrin and fenvalerate), organophosphate (phoxim), and carbamate (carbosulfan) compounds showed higher toxicity to pupae and female adults of parasitoid *M. mediator* than biocides (Bt toxin, BtA, and abamectin). Also, Xu *et al.* (2004) reported that insecticides might affect parasitoids directly by causing mortality or indirectly by impairing their performances. The majority of pesticides applied in the field were reported to be highly toxic to biological control agents. The side-effects of carbamates, phophorates, and pyrethroids on several species of natural enemies and the effects on different biological parameters had been reviewed (Croft, 1992). Carbamates and phosphorates were two classes of insecticides which showed harmful effects on the agroecosystem (Singh and Varma, 1986; Basedow, 1999). Similar experiments to the present study were performed by Talekar and Yang (1991) with another pyrethroid insecticide, deltamethrin. Delamethrin had high toxicity to adults of *A. pultella* and *Diadegma eurcerophaga*, and parasitoids of *P. xylostella*. Most chemical insecticides tested, including pyrethroids which had a low toxicity to the diamondback moth, were highly toxic to its parasitoid (Mani and Krishnamoorthy, 1984; Talekar and Yang, 1991). Idris and Grafius (1993) reported the same trend of the insecticide cypermethrin, which was even harmful to the agroecosystem of vegetable fields by killing parasitoids, causing approximately 100% mortality to the adult stage of another parasitoid of the diamondback moth, *Diadegma insulare* Cress, at the recommended field rate. In contrast, Haseeb *et al.* (2000) reported that recommended field rate of IGRs (chlorfluazuron, teflubenzuron, and flufenoxuron) were found non-toxic to *Diadegma semiclausum* (Hellen) parasitoid of *P. xylostella*. Moreover, Croft (1990) and Entwistle *et al.* (1993) reviewed that the microbial product from *B. thuringiensis* were safe to non-target organisms, and were generally regarded as compatible with biological control agents (Wright and Verkerk, 1995). At the recommended field rate (1 200 mg a. i./L), commercial Bt-insecticides (*e. g.*, Xentari® and Crymax®) were safe to *D. insulare* adults and pupae of *P. xylostella* (Xu *et al.*, 2004). According to this present study, Bt toxin and chlorfluazuron did not show obvious contact toxicity to pupae and adults of *M. mediator*.

Based on these findings, it can be concluded that BtA has much higher selectivity between *M. mediator* and *H. armigera* than the other commercial insecticides. BtA should be included in the developing biological control strategies in crop fields. It was toxic to the lepidopterous pest *H. armigera*, but had a relatively low toxicity to its parasitoid, *M. mediator*. BtA can result in lower percentages of larval mortality and has an increased toxicity against *H. armigera* compared to abamectin alone. BtA gives results comparable to or better than the remaining commercial insecticides in causing larval mortality. BtA has increased efficacy against *H. armigera* compared to Bt alone and is safer to *M. mediator* than abamectin and other chemical insecticides used at the same rate in the laboratory. Therefore, BtA should be integrated into the IPM program for *H. armigera*.

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复配杀虫剂 BtA 对棉铃虫的杀虫效果及对天敌中红侧沟茧蜂蛹和成虫的触杀毒性

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摘要: 为了科学施药, 合理保护和利用天敌对棉铃虫 *Helicoverpa armigera* 进行综合防治, 本研究在室内测定和比较了复配杀虫剂 BtA 和 7 种常用杀虫剂(阿维菌素、Bt、β-氯氰菊酯、辛硫磷、定虫隆、氰戊菊酯和丁硫克百威)对棉铃虫的杀虫效果及对其天敌中红侧沟茧蜂 *Microplitis mediator* 蛹和雌成虫的触杀毒性。采用浸叶法测定杀虫剂在室内 72 h 内对棉铃虫 3 龄幼虫的杀虫效果, 结果表明: 与其他杀虫剂相比, BtA 对棉铃虫幼虫具有更高的毒性和致死效应 (LC_{50} = 0.7364 mg/mL)。将棉铃虫 3 龄幼虫接到用浓度 4 mg/mL 上述杀虫剂分别处理过的大白菜上 24, 48 和 72 h, 发现其死亡率之间存在显著差异($P \leq 0.05$)。但在 72 h 后, BtA 和其他杀虫剂对棉铃虫幼虫的杀虫效果之间并无显著差异($P > 0.05$)。BtA 施药后, 随着时间的延长, 棉铃虫幼虫的死亡率也在增加。另外, 通过杀虫剂对寄生蜂中红侧沟茧蜂蛹和雌成虫的触杀毒性的生物测定发现: 与 β-氯氰菊酯、氰戊菊酯和丁硫克百威相比, BtA 对中红侧沟茧蜂蛹和雌成虫的毒性较低, 对其蛹和雌成虫致死率分别仅为 13.82% 和 7.33%。本研究证明 BtA 对鳞翅目害虫具有中等毒性, 而对寄生蜂中红侧沟茧蜂则具有较低毒性。

关键词: 棉铃虫; 中红侧沟茧蜂; 杀虫剂; 杀生剂; BtA; 毒性; 触杀毒性
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